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Metformin interaction with insulin-regulated glucose uptake, using the *Xenopus laevis* oocyte model expressing the mammalian transporter GLUT4

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Abstract

The primary goal of this work was to better define, in molecular terms, the impact of metformin on hexose carriers. The methodology consisted of determining the zero-trans kinetics of 2-deoxy-D-glucose uptake for the mammalian insulin-sensitive glucose transporter (GLUT4) expressed in *Xenopus laevis* oocytes. These cells possessed the specialized protein and, when treated with insulin (2 μM) plus metformin (20 μM), showed a markedly enhanced hexose transport activity (2.4-fold increase over basal) as compared to that of cells incubated in the presence of insulin alone (1.8-fold increase over basal). Kinetic analysis of this process revealed that insulin induced a similar response to that observed for the native carrier, i.e., a higher V_{max}. When metformin was added together with insulin, we mainly recorded a significant decrease in apparent K_m for the sugar transported, V_{max} being only marginally modified. Parathyroid hormone (PTH), which is known to impair the intrinsic activity of GLUT4, prevented the stimulatory effect of metformin in both kinds of oocytes whereas cytochalasin D, which interferes with the translocation of carriers, was without effect. These results suggest that metformin combined with insulin can maintain glucose homeostasis by increasing the catalytic activity of some hexose carriers or by improving the affinity of GLUT4 for glucose.

Keywords: Metformin; Insulin; *Xenopus* oocytes; Glucose uptake; Parathyroid hormone; GLUT4; Intrinsic activity

1. Introduction

Stimulation of glucose transport into insulin-responsive cells, namely muscle cells and adipocytes, is a pivotal event in the early actions of this hormone (for recent review, see Holman and Kasuga, 1997). Furthermore, under most physiological circumstances, hexose transport represents a rate-limiting step of overall glucose disposal. Since abnormal function of this biological effector is one of the primary features of diabetes, there is a great benefit to be gained from identifying which steps of the glucose uptake process are subjected to regulation by antidiabetic drugs such as metformin. To date, however, relatively little is known about the mechanisms that determine the action of this biguanide on glucose transport at the molecular level (Wiernsperger and Rapin, 1995). Moreover, the respective contribution of the intrinsic properties of hexose carriers vs. their number expressed at the plasma membrane in the hormonal control of the aforementioned uptake remains a question of debate (Zierler, 1998).

Studies performed in vitro on muscle biopsies from diabetic patients (Galuska et al., 1991) or on adipocytes from Zucker rats (Matthaei and Greten, 1991) have shown that metformin improves deficient glucose transport in insulin-resistant tissues when stimulated by insulin. This particular effect of metformin was, however, observed at high drug concentrations and was thought to be due to increased translocation, from an intracellular pool to the plasma membrane, of the glucose transporter subtype activated specifically by the hormone, i.e., GLUT4 (Hundal et al., 1992; Kozka and Holman, 1993). The positive effect of metformin on cells which are mainly or only equipped with transporters insensitive to insulin supports evidence that metformin also increases the intrinsic activity of some membrane-trapped carriers. This result was well demonstrated in the case of erythrocytes, a cell type which has only GLUT1 (Yoa et al., 1993; Wiernsperger, 1996). Other

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studies performed with adipocytes and muscle cells have similarly confirmed this pharmacological activity of metformin, i.e., a greater transport capacity of carriers when bound to the cell membrane or a normalization of this process in diabetic conditions (Klip and Leiter, 1990; Matthaei et al., 1993; Thomas et al., 1998).

These findings reveal that metformin closely interacts with mechanisms involving the hexose transporters but we do not know exactly what factors determine the translocation or improved transport capacity of a specific protein and how the hormonal signal is modulated. Recently, we highlighted the advantages of using the *Xenopus laevis* oocyte model to unravel the potentiating effect of metformin on glucose metabolism (Detaille et al., 1997, 1998). In particular, we found that the drug at a therapeutical dose (20 μM) stimulated both the uptake of D-glucose and 3-*O*-methyl-D-glucose induced by insulin (2 μM) in oocytes possessing their own transporters. In addition, the ability of these cells to easily translate foreign mRNAs introduced by microinjection makes the oocyte system an ideal tool for the analysis of heterologously expressed proteins (Heikkila, 1990). Moreover, *Xenopus* oocytes contain abundant functional insulin (as well as insulin-like growth factor I) receptors while exhibiting relatively low endogenous glucose transport rates (Janicot and Lane, 1989; Thomson et al., 1997). Therefore, the use of this cell type as an expression system, unlike many mammalian cells which express more than one isoform of hexose carrier, is a reliable approach to solve the current issue despite the weak sensitivity of these cells to insulin.

Here, we firstly describe the kinetics of both the endogenous oocyte carrier and the mammalian transport protein GLUT4 expressed in *Xenopus* oocytes, after the combined action of insulin and metformin. Parathyroid hormone (PTH) was recently found to abolish the insulin-stimulated dephosphorylation of GLUT4 in adipocytes (Reusch et al., 1993), thereby impairing its intrinsic activity without any alteration in its cellular distribution or translocation. Another substance, cytochalasin D, also inhibits insulin-stimulated glucose transport by preventing the recruitment of GLUT4 via a disassembly of the actin network (Tsakiridis et al., 1997). Given these observations, we attempted to elucidate in what way metformin, when added with insulin, is able to effect changes in each expressed transporter isoform by monitoring the transport of labeled 2-deoxy-D-glucose in both uninjected and injected oocytes in response to PTH or cytochalasin D treatment.

2. Materials and methods

2.1. Materials

Metformin hydrochloride was a gift from LIPHA (Lyon, France). Porcine insulin, 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, gentamicin sulfate, cytochalasin D and phloretin were from Sigma (St. Louis, MO. USA). 2-Deoxy-D-[1-^{3}H]glucose (11 Ci/mmol) and 3-0-methyl-D-[*U*-^{14}C]glucose (103 mCi/mmol) were purchased from Amersham. PTH was from Calbiochem-Novabiochem (San Diego, CA, USA). The cDNA encoding the rat adipocyte glucose transporter GLUT4 has been previously described (James et al., 1989) and was generously provided by Dr. Mike Mueckler (Washington University Medical School, MI, USA).

2.2. Synthesis of transcripts from cDNA

Plasmids containing the cDNA insert encoding rat adipocyte GLUT4 were linearized by *Hin*dIII restriction-endonuclease digestion. The DNA was recovered by phenol extraction followed by ethanol precipitation and then transcribed in vitro into capped RNA, using T7 RNA polymerase and an mRNA capping kit from Stratagene (La Jolla, CA, USA). Complementary RNA (cRNA) was recovered by precipitation with ethanol plus sodium acetate and finally dissolved at a concentration of 4 μg/μl in sterile diethylpyrocarbonate-treated buffer. The yield of the in vitro transcription product was quantified by spectrophotometry at 260 nm and transcript integrity was checked on a formaldehyde-agarose gel.

2.3. Preparation of Xenopus oocytes and RNA injection

All procedures concerning the handling of the oocytes as well as their microinjection have been extensively described in a previous paper (Detaille et al., 1998). Briefly, after the individual mature oocytes (stages V and VI) were washed with modified Barth's saline (MBS), they were isolated free of follicular cells and incubated overnight at 4°C in MBS with 100 μg/ml gentamycin sulfate until injection. Healthy-looking oocytes were visually selected and injected with 20 nl of a RNA solution (2 μg/μl) or sterile water at the border of the vegetal hemisphere. The injected oocytes were then maintained at 22°C for 3 to 5 days in 5 ml of MBS containing gentamicin sulfate before assaying glucose uptake. The incubation medium was changed after 1 day and the degenerating oocytes were discarded.

2.4. Glucose uptake measurement

A few days following the injection of the appropriate cRNA, groups of 8 to 16 oocytes were directly transferred into 2 ml of MBS alone or MBS containing 2 μM insulin in the absence or presence of 20 μM metformin. After incubation for 90 min at room temperature, 2-deoxy-D-[1-^{3}H]glucose was added at various concentrations for 10 more minutes. The reaction was stopped by quickly removing the radioactive solution and washing the oocytes in 3×5 ml of ice-cold phosphate-buffered saline (PBS) with 0.3 mM phloretin. The cells were then lysed in 1 ml of

Table 1
Summary of kinetic parameters for the transport of sugars into *X. laevis* oocytes possessing only endogenous carriers. K_m and V_{max} values were calculated from Lineweaver–Burk plots such as those shown in Fig. 4. All results are means ± S.E.M. from three to five separate experiments for each condition

Conditions	Substrate	K_m (mM)	V_{max} (pmol/min/oocyte)
Basal (MBS alone)	2-deoxy-D-glucose	1.9 ± 0.17	27.2 ± 4.7
	3-*O*-methyl-D-glucose	1.7 ± 0.25	32.4 ± 5.3
Metformin (20 μM)	2-deoxy-D-glucose	2.0 ± 0.21	28.3 ± 6.3
	3-*O*-methyl-D-glucose	not determined	not determined
Insulin (2 μM)	2-deoxy-D-glucose	2.1 ± 0.18	56.9 ± 8.8
	3-*O*-methyl-D-glucose	2.2 ± 0.24	66.8 ± 4.5
Metformin (20 μM) + Insulin (2 μM)	2-deoxy-D-glucose	2.2 ± 0.17	77.2 ± 6.0
	3-*O*-methyl-D-glucose	2.3 ± 0.29	85.8 ± 4.3

sodium dodecyl sulfate (SDS) 1.5% or 0.05 M NaOH and the amount of glucose taken up by oocytes, injected or not, was calculated after liquid scintillation counting. Another structural analog, 3-*O*-methyl-D-glucose, was also used in some experiments. For the studies which were intended to target an action of metformin linked to the functioning of each carrier (translocation vs. intrinsic activity), an additional 60-min preincubation with PTH or a longer incubation (2 h) with cytochalasin D, used at a final dose of 10 nM and 25 μM, respectively, was made before adding insulin combined or not with metformin during the next 90 min as described above.

The experimental parameters were selected to ensure that the assays were carried out under conditions of initial velocity (zero-trans transport), thus allowing the determination of kinetic constants. It is worth mentioning that zero-trans kinetics are more easily determined with 2-deoxy-D-glucose than with non-phosphorylatable derivatives such as 3-*O*-methyl-D-glucose since it is trapped in the cell after transport. Moreover, the rank-order affinity for 2-deoxy-D-glucose transport as determined by the zero-trans method was identical to that measured by equilibrium exchange using 3-*O*-methyl-D-glucose (Gould et al., 1991). Data are presented as means ± S.E.M. and statistical dif-

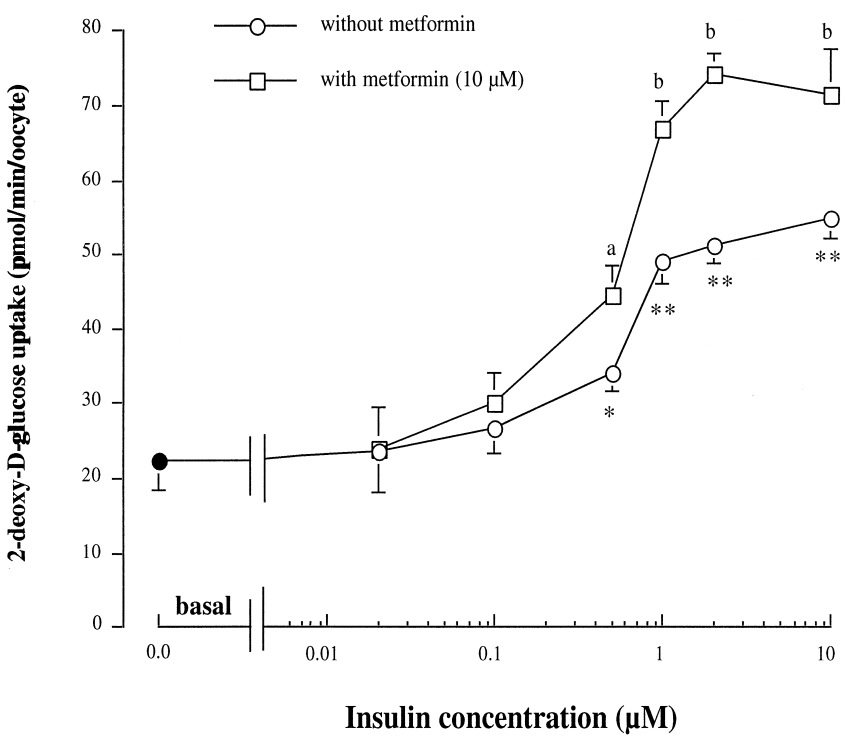

Fig. 1. Effect of metformin on the insulin-mediated increase in 2-deoxy-D-glucose transport by native *Xenopus* oocytes. Groups of 20 oocytes were transferred to 1 ml of MBS containing the indicated micromolar concentrations of insulin (ranging from 0.02 to 10 μM). After incubation for 90 min in the absence or presence of metformin (10 μM), the cells were lysed before determining, in a 10-min standard assay, the initial rates for hexose (4 mM) uptake. The results are the means ± S.E.M. ($n = 4$). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. basal transport. $^{a}P < 0.01$, $^{b}P < 0.005$ vs. insulin-induced transport.

ferences were determined by Student's *t*-test. A probability of 5% or less was accepted as significant.

3. Results

Preliminary experiments with native *Xenopus* oocytes confirmed the enhancing effect of metformin on insulin-induced glucose uptake. It was characterized by an additional increase (30%, $P < 0.05$) in the maximal capacity of carriers (V_{max}) without affecting the affinity (K_m) for hexose (Table 1). The biguanide drug combined with insulin also augmented the uptake of the non-metabolizable sugar 3-*O*-methyl-D-glucose to the same extent as 2-deoxy-D-glucose, suggesting clearly a stimulation of transporters pre-existing at the oocyte cell surface. Metformin did not appear to alter qualitatively the dose–response effect exerted by insulin upon glucose transport into these native oocytes. Although the simultaneous presence of metformin during the preincubation step changed the level of activation (from a 2.2-fold increase with insulin alone to a 3.3-fold increase in the presence of insulin plus metformin), no modification of the concentration of insulin necessary to induce the uptake process was observed (Fig. 1). Maximal activation was achieved with 2 μM insulin, with an ED_{50} of about 0.5 μM.

Xenopus oocytes also expressing the mammalian facilitative transport protein GLUT4 showed a markedly enhanced capacity to take up 2-deoxy-D-glucose, when added at a final dose of 1.8 mM (a 3.5-fold increase over basal transport activity in the native cells, $P < 0.005$). No significant effect was recorded in the presence of metformin alone (Table 1; Fig. 2). The degree of glucose uptake in such oocytes, by comparison with the uninjected ones, augmented from a 1.7-fold increase when treated with insulin to a 2.4-fold increase when treated with metformin plus insulin. These observations therefore mean that the insulin sensitivity of the newly synthesized transporter is similar to that of the endogenous oocyte carrier since we measured under both conditions a doubling of 2-deoxy-D-glucose uptake after exposure of *Xenopus* eggs to 2 μM insulin ($P < 0.01$). The efficiency of metformin tended to be slightly more pronounced with the exogenous carrier than with the native proteins (1.57- vs. 1.25-fold increase). Nonetheless, the drug effect combined with hormonal induction was statistically the same (significant at $P < 0.05$)

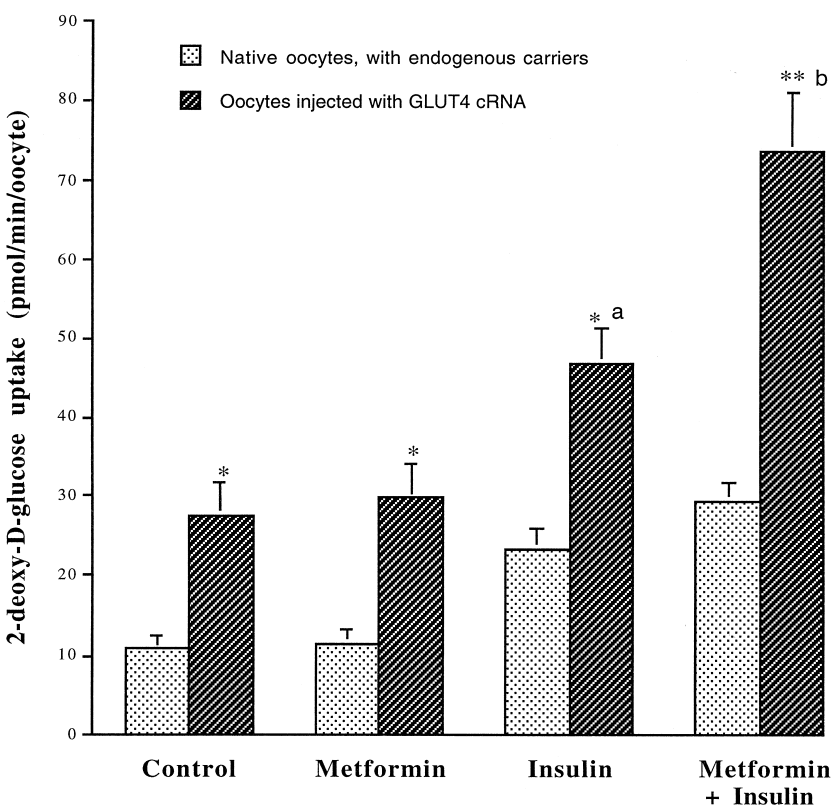

Fig. 2. Comparative effect of metformin on glucose transport in native oocytes and in oocytes expressing the mammalian glucose transporter GLUT4. Mature oocytes were first microinjected with the in vitro synthesized RNA encoding the rat adipocyte protein GLUT4 (15 ng per oocyte). Assays were performed 3 days after injection. Before hexose uptake was measured, both uninjected and injected oocytes were incubated for 90 min in MBS alone, in MBS containing metformin (20 μM) and in MBS containing the biguanide combined or not with insulin (2 μM). The final concentration of sugar in the medium was 1.8 mM. The results are the means ± S.E.M. ($n = 5$, each performed in duplicate). $^{*}P < 0.005$ or $^{**}P < 0.001$ denotes a statistically significant increase in 2-deoxy-D-glucose uptake compared with that of native oocytes. [a]Insulin effect significant at $P < 0.01$ (compared to basal conditions without metformin). [b]Metformin effect coupled with hormonal induction significant at $P < 0.05$.

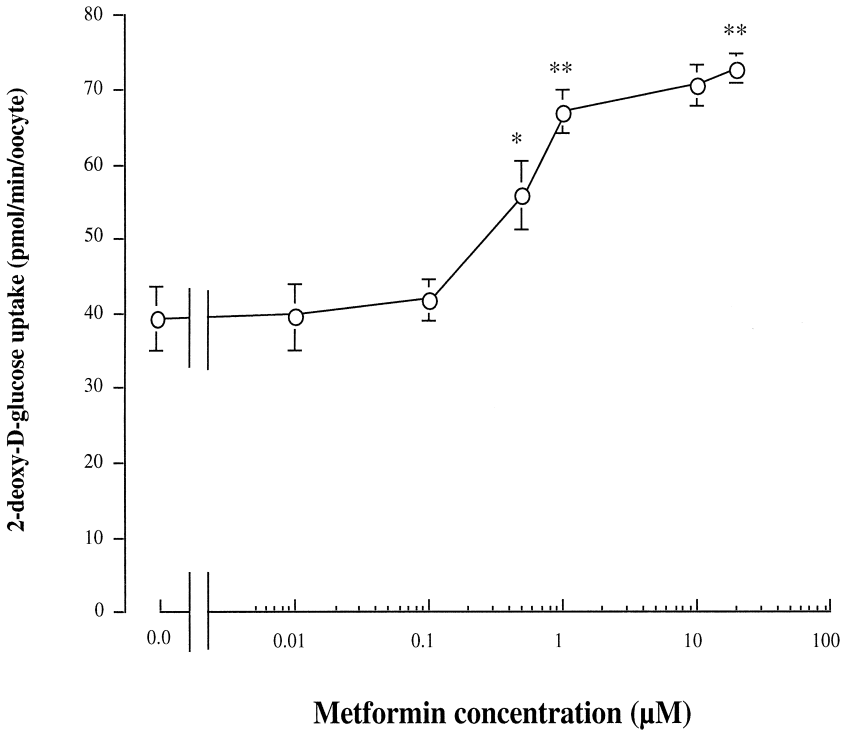

Fig. 3. Dose–response curve for the effect of metformin on 2-deoxy-D-glucose uptake by oocytes expressing the mammalian glucose transporter GLUT4. The oocytes were incubated as described in the legend of Fig. 2 unless they were maintained in MBS containing insulin (2 μM) with increasing concentrations of metformin (up to 20 μM) for 90 min. After these treatments, the uptake of labeled hexose (1.8 mM) was measured as reported previously. The results are the means ± S.E.M. ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.02$ (both vs. insulin effect alone). Transport activity under basal conditions was 26.6 ± 3.3 pmol 2-deoxy-D-glucose per min and per oocyte.

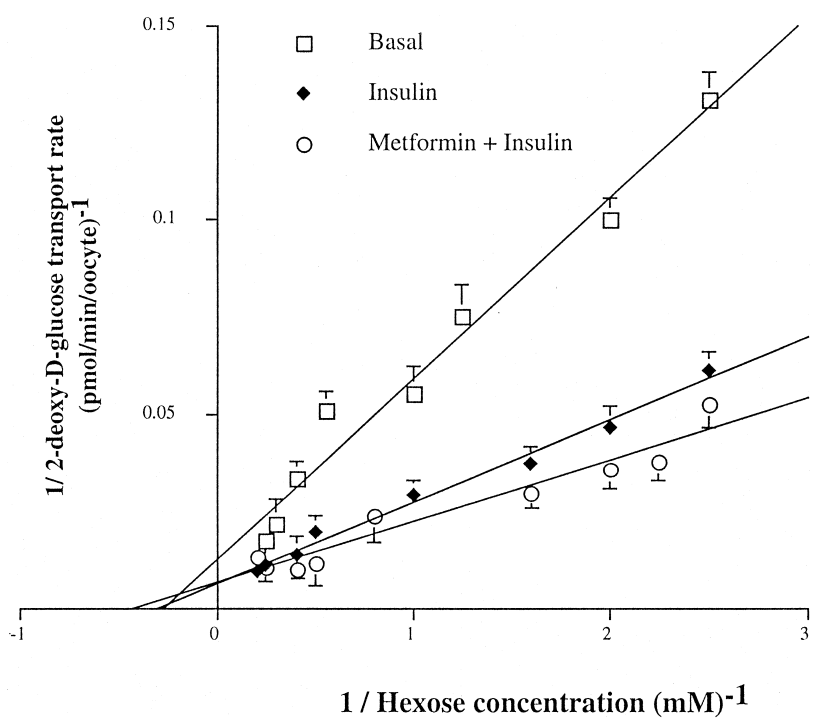

Fig. 4. Dependence of 2-deoxy-D-glucose transport activity upon hexose concentration in microinjected *Xenopus* oocytes. The oocytes were processed and preincubated in the presence of test molecules as described in Fig. 2. Initial rates of hexose uptake were then measured in a 10-min standard assay, using three to five groups of 12 oocytes per point. The data were linearized and plotted according to the Lineweaver–Burk's representation.

whatever the group of cells studied. Very interestingly, metformin together with 2 μM insulin stimulated glucose transport mediated by the carrier GLUT4 in a dose-dependent manner (Fig. 3). The biguanide induced maximal

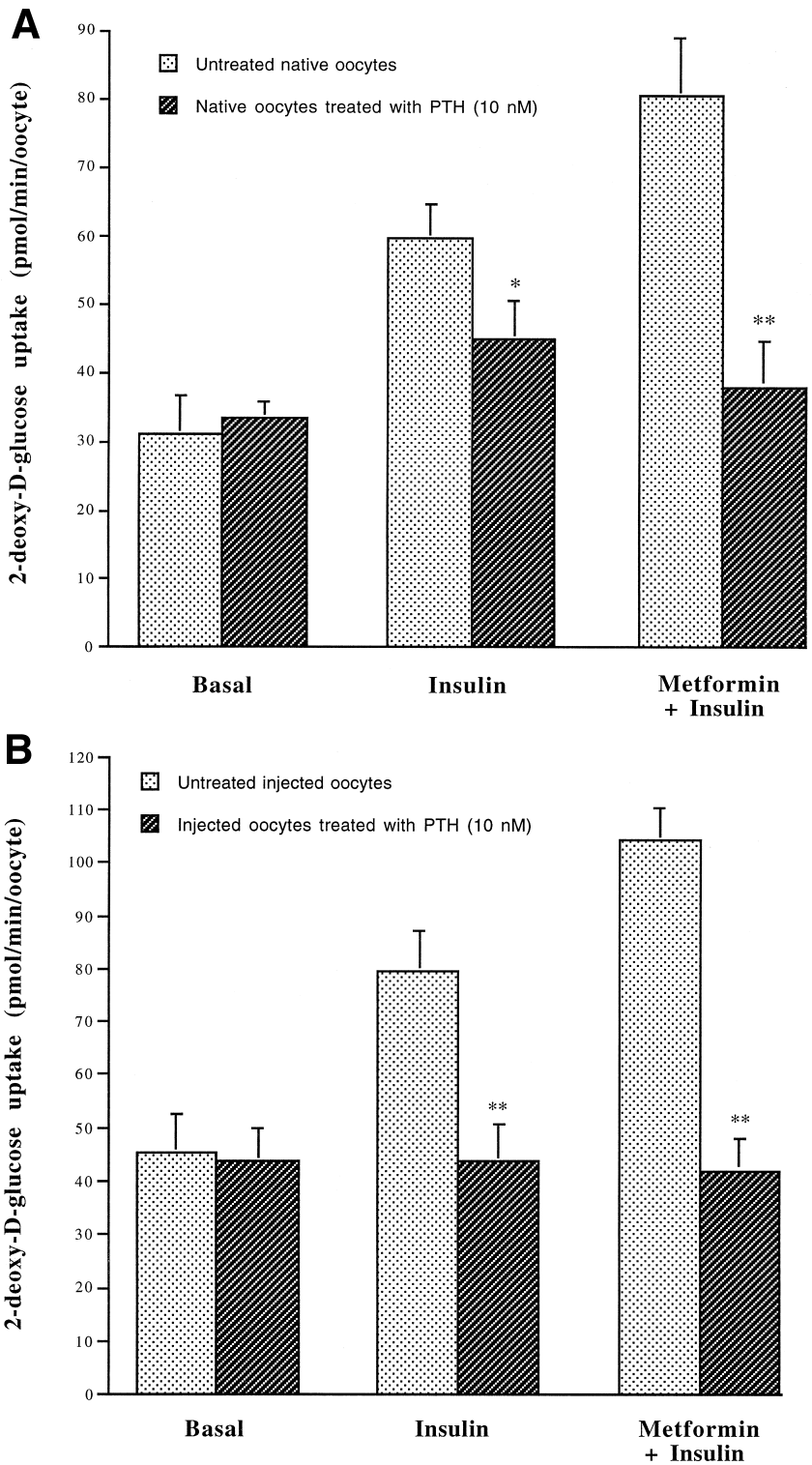

Fig. 5. Effect of PTH on basal 2-deoxy-D-glucose transport or on insulin-stimulated transport in the absence or presence of metformin. Native oocytes (panel A) as well as oocytes injected with 15 ng of RNA encoding rat adipocyte protein GLUT4 (panel B) were first incubated in MBS containing PTH at a final dose of 10 nM for 60 min. Insulin alone (2 μM) or insulin plus metformin (20 μM) was added and both groups of oocytes were further incubated for 90 min prior to the measurement of hexose uptake. The final concentration of 2-deoxy-D-glucose in the medium was 5 mM (A) or 1.8 mM (B). The results are the means ± S.E.M. ($n = 3$ to 6) $^{*}P < 0.02$, $^{**}P < 0.001$ vs. effect of insulin alone or combined with metformin, in native oocytes. $^{*}P < 0.005$ vs. insulin effect, in the absence and in the presence of the drug, on the oocytes expressing GLUT4.

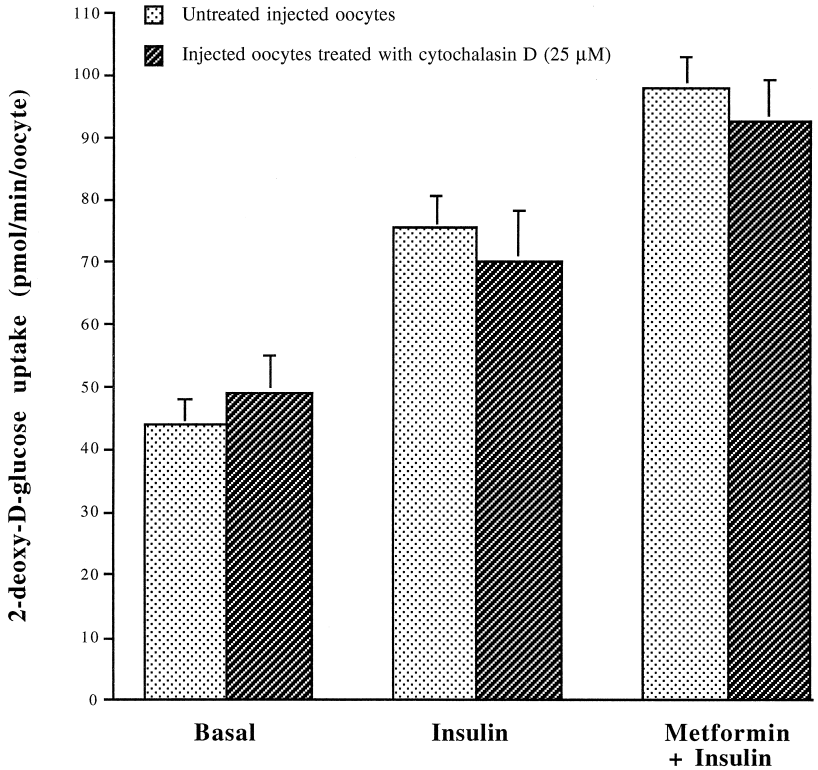

Fig. 6. Effect of cytochalasin D on basal 2-deoxy-D-glucose transport or on insulin-stimulated transport in the absence or presence of metformin. Cells expressing the protein GLUT4 were pretreated with cytochalasin D (a potent disrupter of the actin cytoskeleton) for 2 h at a final dose of 25 μM. Insulin alone (2 μM) or in association with metformin (20 μM) was then added and the oocytes were further incubated for 90 min prior to the measurement of hexose uptake as described previously. The final concentration of 2-deoxy-D-glucose in the medium was 1.8 mM. The results are the means ± S.E.M. ($n = 5$).

activation of uptake at 20 μM, with a half-maximal response at about 0.4 μM.

To next determine whether the functional activation of GLUT4 caused by metformin corresponded with significant changes in the kinetic constants for hexose, the dependence of 2-deoxy-D-glucose uptake on sugar concentration was investigated in a zero-trans uptake assay. We found (Fig. 4) that insulin alone had little, if any, effect on the apparent K_m for 2-deoxy-D-glucose (3.08 ± 0.11 vs. 3.2 ± 0.32 mM) but induced a significant increase in V_{max} (133.9 ± 10.3 vs. 72.8 ± 4.9 pmol/min/oocyte, $P < 0.01$). Conversely, metformin together with insulin did not elicit any further increase of V_{max} (125.0 ± 10.7 vs. 133.9 ± 10.8 pmol/min/oocyte) but instead appeared to reduce the K_m value for hexose (2.44 ± 0.26 vs. 3.08 ± 0.11 mM, $P < 0.05$), suggesting that this drug in close association with the hormone could increase the affinity or even the transport capacity of GLUT4. This hypothesis was, at least indirectly, confirmed by measuring the transport activity in both kinds of oocytes pretreated with PTH or cytochalasin D. PTH (10 nM) totally eliminated the stimulating action of metformin ($P < 0.005$ for the native cells, $P < 0.001$ for the injected ones), whether the final concentration of 2-deoxy-D-glucose in the medium was 5 mM (Fig. 5A) or 1.8 mM (Fig. 5B), i.e., respectively, higher and lower than the K_m value. The effect of insulin alone was also prevented, but to a lesser extent especially in native oocytes (Fig. 5A). Unlike PTH, cytochalasin D (25 μM) failed to inhibit glucose transport stimulated by insulin in the presence or absence of metformin in injected oocytes (Fig. 6). We found similar results for uninjected oocytes (data not shown).

4. Discussion

We and others recently pointed out the relevance of the *X. laevis* oocyte model for investigating the mode of action of a drug, as demonstrated in the case of metformin (Grigorescu et al., 1991; Stith et al., 1996; Detaille et al., 1998). We previously established that the presence of insulin was absolutely required to observe an effect of this biguanide on glucose uptake and decided to further study the relationship between the molecular effects of insulin and those of metformin, using as experimental tool oocytes expressing the mammalian protein GLUT4, i.e., the hormone-sensitive hexose transporter. It is worth recalling that this cell type was chosen because of the ease with which the expression of a single transporter isoform from foreign mRNA microinjected is achieved.

The mechanism by which insulin stimulates glucose transport in fat and muscle cells has been the focus of much work owing to the clinical relevance to diabetes mellitus (Kahn, 1992). On the basis of numerous experiments with these somatic cells, it is usually considered that insulin predominantly causes the recruitment of GLUT4 carriers from an intracellular storage pool to the plasma membrane, where increased transport is measured as a result of an increased V_{max} (Cushman and Wardzala, 1980; Zorzano et al., 1989; Handberg, 1995). Nevertheless, whether this is a unique process of transport activation is still somewhat controversial since some authors reported that the degree of translocation was often smaller than the rate of insulin-enhanced sugar uptake (Joost et al., 1988; Czech et al., 1992). The concept has therefore been raised that insulin and probably other factors modulate the intrinsic activity of the hexose carriers GLUT4 and GLUT1 present in various target cells, with intrinsic activity being defined as the transport capacity or kinetic turnover number, i.e., V_{max} divided by K_m (Sargeant et al., 1993). Our preliminary kinetic studies with *Xenopus* oocytes containing native transporters, immunologically identified as GLUT1-like carriers (Hainaut et al., 1991), showed that metformin in the presence of insulin increased the V_{max} value for 2-deoxy-D-glucose transport. The likelihood that insulin, whether metformin was present or not, promotes some translocation of the endogenous carrier is greatly weakened by the results obtained with PTH (inhibitory effect, see Fig. 5) and cytochalasin D (no effect). From these observations, it is reasonable to consider that the native carrier residing inside the plasma membrane of stimulated oocytes exhibits a mode of regulation by metformin which is quite similar to that involving the typical transporter GLUT1 present in erythrocytes (Yoa et al., 1993). Although these cells (and, a fortiori, their own GLUT1) do not respond to insulin, metformin exerted its beneficial action on glucose uptake through an increase in V_{max} but not in K_m. A potential explanation for this higher V_{max} is that a significant proportion of GLUT1 transporters exist in an inactive conformational state or in a masked form inside the plasma membrane of unstimulated cells, as recently reported (Zhang and Ismail-Beigi, 1998), and that treatment with metformin in the presence of insulin could lead to the apparent activation of these transporters.

In the case of oocytes expressing the protein GLUT4, the translocation component did not fully account for our data since insulin elicited an approximately twofold increase in V_{max}, comparable (or even inferior) to that measured in uninjected *Xenopus* oocytes. That the hormonal responsiveness of the added GLUT4 carrier was similar to that observed for the endogenous protein is in complete accordance with previous findings from reports which claimed that the activation of a given hexose transporter is dependent on the cellular context or membrane environment in which the protein is expressed (Keller et al., 1989; Vera and Rosen, 1989). Besides, the requirement of high insulin concentrations (in the micromolar range) to produce a maximal effect on glucose transport is probably a property inherent to oocytes and not specifically related to the glucose transporter. This is also consistent with the notion that these cells have very few high-affinity insulin receptors (Vera and Rosen, 1990). Considering the magnitude of the changes in basal and insulin-induced transport uptake, it is thus tempting to suggest that a fraction of the newly introduced GLUT4 transporters are directly targeted or translocated to the cell surface independently of any hormonal action. We cannot exclude the fact that a substantial proportion of these carriers are sequestered into an intracellular compartment of the *Xenopus* oocyte, as reported by Marshall et al. (1993). However, it is unlikely that insulin mobilises them (or if it does, this mechanism was ineffective under our conditions) in order to markedly increase hexose uptake into injected oocytes. Our results are in striking contrast with recent data showing that both insulin and insulin-like growth factor I stimulate the recruitment of GLUT4 with a concomitant increase in glucose transport activity into oocytes (Mora et al., 1995). Nevertheless, at this point, it should be emphasized that the participation of GLUT4 in this phenomenon is not unequivocally observed (Burant and Bell, 1992) and that insulin has also been found to be without effect on glucose transport in oocytes expressing these carriers (Thomas et al., 1993).

Kinetic analysis of transport in oocytes expressing the protein GLUT4 demonstrated, for the first time, the capacity of metformin to increase the affinity of a glucose carrier for its substrate since we mainly recorded a marked reduction of the apparent K_m (see Fig. 4). Nevertheless, taking into account the results with PTH, namely a full blockade of the enhancing effect of metformin on glucose transport hormonally induced in both kinds of oocytes, and considering the definition of intrinsic activity given earlier, it seems likely that the changes in the kinetic parameters (either an increase of V_{max} or a decreased K_m) essentially reflect a modulation of the intrinsic transporting ability of glucose carriers, i.e., GLUT1 and GLUT4 rather than the additional appearance of these proteins at the cell surface. The lack of inhibition by cytochalasin D strongly corroborates this hypothesis. Likewise, the dose–response curve for the effect of metfomin on GLUT4-mediated glucose uptake (see Fig. 3) was consistent with the typical description of a receptor induced-like event and supports the concept of drug-induced modulation of intrinsic activity.

From our data set, it can be inferred that, after stimulation by insulin, the adipocyte glucose transporter expressed in the *X. laevis* oocyte displays a kinetic behavior close to that of the native carrier of oocytes. The latter does not moreover exhibit the classical features of GLUT1 in terms of insulin sensitivity. Although the current experiments do not permit an easy discrimination between direct and indirect effects of metformin on the glucose transporter system, we strongly believe that the stimulatory action of

this biguanide in the presence of insulin is mediated by functional activation of carriers (already present in the membrane), as reflected by modification of the ratio V_{max}/K_m. This probably means that metformin facilitates glucose entrance across the lipid bilayer either by modulating the catalytic properties of native or newly expressed carriers or by increasing more specifically the affinity of GLUT4, rather than by promoting carrier redistribution.

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